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A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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INCREASED RED BLOOD CELL FRAGILITY AFTER FAT INGESTION[1]

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There have been indications that ingested fat might be injurious to red blood cells. In studies on relatively few human individuals over brief periods of time, red blood cell destruction apparently proceeded at a faster rate on a high fat diet, as evidenced by an increased urobilin excretion in the feces (Glanzmann, 1929; Salomon, 1920; Josephs, Holt, Tidwell and Kajdi, 1938). The experiments of Freeman, Loewy, Marchello, and Johnson (1942) carried out for many weeks conclusively demonstrated this effect by the more direct method of measurement of the bilirubin excretion in dogs, which was greater with increases of fat in the diet.

Some of the mechanisms involved in this blood destruction from fat ingestion were clarified by Johnson and Freeman (1938), who showed that during the absorption of a fat meal, lymph collected from the lacteals or from the thoracic duct is very hemolytic. It was also demonstrated (Freeman and Johnson, 1940) that fatty acids and soaps, which have presumably escaped resynthesis into neutral fat during absorption, are present in the lymph in quantities sufficient to account for this hemolysis.

Since the lymph entering the blood after a fat meal is hemolytic, more direct evidence of red blood cell injury in the circulating stream at that time was sought. In preliminary experiments on dogs, actual *hemolysis* did not occur when red blood cells were mixed with lipemic serum drawn after a fat meal. The experiments here reported test whether red cells are made more *fragile* by exposure to such serum.

Methods and results. The fragility of red cells mixed with lipemic serum was compared with that of cells similarly mixed with fasting serum. The fragility test involved identical dilutions with distilled water of both sets of blood plus serum mixtures, gentle shaking of these diluted mixtures, and finally, counting the intact cells (per cubic millimeter) in each sample. Addition of the water reduced the inorganic salt concentration to approximately the equivalent of 0.4 per cent NaCl, midway between the concentrations producing beginning and total hemolysis in dogs.

Each dog tested was fed a fat meal consisting of 10 cc. of olive or corn oil per kilogram body weight in addition to commercial canned dog food. Samples of fasting serum ("control fluid") and of lipemic serum (one of the "test fluids") from the same dog drawn at two and at three and one-half hours after a fat meal were each mixed with an equal volume of whole blood (oxalated) from the fasting

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

animal. After shaking each mixture for forty-five minutes, distilled water was added and the shaking was continued for thirty minutes. The final mixture contained one volume of red cells (i.e., oxalated blood), one volume of fasting or lipemic serum, and two volumes of distilled water. Ordinary red blood cell counts were then done on each mixture.

To express the results graphically (fig. 1), the red cell count of the control mixture (e.g. fasting serum and oxalated blood) is arbitrarily placed at zero hemolysis; that is, the hemolysis from distilled water and from shaking is disregarded since this is identical in both control and test mixtures. The test

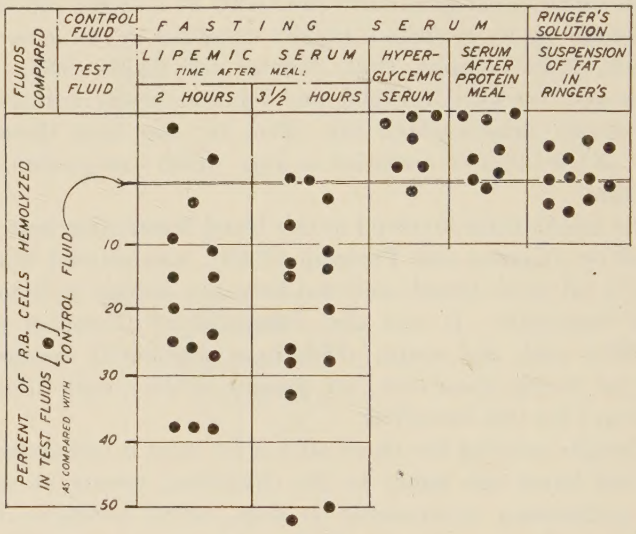

Fig. 1. Change in fragility of red blood cells upon exposure to various test fluids. The test counts are plotted as per cent reduction (the ordinate) from the corresponding control count which is represented by the zero line. To avoid confusion, these control points are omitted.

count is then expressed as percentage reduction from the control. In other words, it represents the increase in hemolysis caused by the test fluid.

When lipemic serum was the test fluid, red cell fragility was significantly increased, as indicated by the lower counts in the test mixtures analyzed statistically. There was no appreciable difference between the effects of the two and the three and one-half hour sera. The average reduction from the control for all lipemic sera tested was 19 per cent.

To check on the possibility that ingestion of other fuel foods might increase red cell fragility, high sugar and high protein meals were fed. The hyperglycemic serum and the serum after the protein meal, respectively, were used as test fluids, substituting for lipemic serum in procedures exactly parallel to those employed with high fat meals. No increase in the fragility of red cells exposed

to these sera was observed. If anything, the *resistance* of the red cells was increased in these caloric controls as indicated by the higher counts in the test mixtures (see third and fourth columns of fig. 1). However, too few experiments have been performed to warrant any final conclusion on this point. Earlier workers have reported that large quantities of sugar do exert a protective effect on red cells preserved *in vitro* (Rous and Turner, 1916). Also, insulin hypoglycemia has been shown to increase the fragility of red cells (Booth, 1941).

Although other studies (Freeman and Johnson, 1940) have shown it to be unlikely that the neutral fat particles in any way affect the fragility of red cells, it was felt worthwhile to test the possibility under the conditions of this experiment. A stable suspension of olive oil in Ringer's solution was used as the test fluid; the particle size and the concentration approximated those in lipemic serum. This was compared with Ringer's solution as the control fluid. Neutral fat was found to be as ineffective as Ringer's solution in altering red cell fragility (see last column of fig. 1). The deviation here was from −6 per cent to +5 per cent, perhaps giving a good indication of the limits of error of the method.

Discussion. These experiments demonstrate the presence in dogs' lipemic serum of one or more agents which act directly on red blood cells to increase their fragility. Probably these agents are the free fatty acids and soaps which account for the hemolytic potency of thoracic duct lymph after fat absorption (Freeman and Johnson, 1940). They apparently proceed from the lymph into the blood in sufficient concentration to cause this increase in red cell fragility. Chemical determinations of these substances in the serum after a fat meal have not been done.

The evidence strongly suggests that red cells are injured *in vivo* very soon after the products of fat digestion enter the bloodstream. It follows that the increased blood destruction during a prolonged high fat diet (Freeman, Loewy, Marchello and Johnson, 1942) may be accounted for largely or wholly by continual repetitions of this short-time effect rather than by some longer, more indirect, metabolic process. Indeed the possibility that ketone bodies may be the destructive agents has probably been eliminated in the experiments of Josephs, Holt, Tidwell and Kajdi (1938). Experiments by Freeman, Loewy and Johnson (1943) lend strong support to the concept that free fatty acids or soaps can act almost at once to increase red cell destruction. They demonstrated an increased bilirubin excretion in anesthetized dogs within an hour or two after injection of small quantities of fatty acid or soap into the jugular vein.

It may be, as perhaps suggested by the experiments where sugar and protein were fed, that the non-fat elements of the diet serve in small part to protect against the injurious effect of fat on the red blood cells. However, at least in dogs, fat ingestion probably is an important mechanism in the normal daily red cell destruction.

Considered in the light of this property of ingested fat, it seems probable that the following well-known phenomena have definite adaptive values hitherto not emphasized: 1, the tendency to vomit after a high fat meal; 2, the slower emptying time of the stomach after fat meals than after meals high in carbohydrate or protein; and 3, the absorption of fat into the lymphatic system and its subsequent gradual introduction into the circulating blood. All these prevent the digestion products of ingested fat from entering the blood stream too quickly or in too great amounts. They would appear to be part of a physiological system of checks to prevent excessive red cell destruction. The ultimate check against anemias of increased red cell destruction is the bone marrow which, in animals on prolonged fat diets, is capable of replacing the extra cell losses (Freeman, Loewy, Marchello, and Johnson, 1943). When these checks are inadequate or when the blood cells themselves are more susceptible to fat injury, an anemia may well develop. These possibilities remain to be investigated as causative factors in certain human anemias.

It is of interest that injury from fat ingestion is encountered in cells other than the erythrocytes. A number of recent animal experiments indicate that high fat diets also produce liver damage. These experiments fall into two groups: 1, those in which fat ingestion apparently damages the liver cells directly (Blumberg and McCollum, 1941; György and Goldblatt, 1941); and 2, those in which fat ingestion acts by increasing the susceptibility of the liver to damage by other agents. Deleterious agents which have been used in such experiments are chloroform (Goldschmidt, Vars, Raudin, 1939), carbon tetrachloride (Bollman, 1940) and arsphenamine (Messinger and Hawkins, 1940). In the case of arsphenamine poisoning, when high carbohydrate or protein diets were changed to high fat diets, the icterus index showed a progressive increase. The work presented here suggests that this icterus may be caused in part by increased blood destruction as well as by liver damage. If fat is injurious to both blood cells and liver cells, it would seem to increase the likelihood that fat ingestion may be related to the etiology of certain anemias.

SUMMARY AND CONCLUSIONS

1. After fat ingestion an agent which increases the fragility of red blood cells is present in dog's serum.

2. Neutral fat is not this agent. Previous work indicates that free fatty acids and soaps, reaching the blood stream by way of the lymphatics, are probably responsible for the effect.

3. Ingestion of carbohydrates and proteins does not increase red cell fragility. On the contrary, meals rich in these substances produce serum which may actually protect red cells from water hemolysis.

4. These results, when considered with the finding of increased red cell destruction *in vivo* on a high fat diet, indicate that fat ingestion probably is an important mechanism in the physiological destruction of red cells in dogs.

5. These experiments suggest further that the mechanism of the demonstrated *in vivo* red cell destruction by fat ingestion is immediate and direct and may not involve any relatively long metabolic process.

6. It is suggested that those physiological mechanisms which delay the absorption of fat and its entrance into the bloodstream are probably significant in the prevention of anemia.

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